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High Fidelity Polymerase Reagent.

Psp polymerase and Tli polymerase are high fidelity polymerase with a fidelity rate that was originally underestimated by New England Biolabs in the early 1990s and reported to be 5x better than Taq when originally marketed. Recent high throughput NGS sequencing has determined that the error rate of Psp polymerase is 50x and the original quantitation was off by one order of magnitude. High fidelity polymerase reagents with a fidelity rate higher than 50x are not a product of a better engineered protein. Instead, a cocktail of various proteins formulated with the reagent produce higher efficiencies in terms of extension time, yield and fidelity. A fusion with Sso7d or a dsDNA binding protein like ETSSB produces quicker extension times higher yield. The inclusion of a UTPase like P45 increases yield by reducing uracil levels and preventing premature extension due to aberrant uracil incorporation. XthA is a 3′-5′ exonuclease that increases fidelity by increasing the de facto processivity of error correction functionality. Below are the proposed proteins that will be included in the dVent purified protein polymerase reagent.

>P45 UTPase
MLHHVKLIYATKSRKLVGKKIVLAIPGSIAAVECVKLARELIRHGAEVHAVMSEAATKIIHPYAMEFATGNPVITEITGFIEHVELAGEHENKADLILVCPATANTISKIACGIDDTPVTTVVTTAFPHI
PIMIAPAMHETMYRHPIVRENIERLKKLGVEFIGPRIEEGKAKVASIDEIVYRVIKKLHKKTLEGKRVLVTAGATREYIDPIRFITNASSGKMGVALAEEADFRGAEVTLIRTKGSVKSFVENQIEVETV
EEMLSAIENELRSKKYDVVIMAAAVSDFRPKIKAEGKIKSDRSITIELVPNPKIIDRIKEIQPNVFLVGFKAETSKEKLIEEGKRQIERAKADLVVGNTLEAFGSEENQVVLIGRDFTKELPKMKKRELA
ERIWDEIEKLLS*

>ETSSB
MEEKVGNLKPNMESVNVTVRVLEASEARQIQTKNGVRTISEAIVGDETGRVKLTLWGKHAGSIKEGQVVKIENAWTTAFKGQVQLNAGSKTKIAEASEDGFPESSQIPENTPTAPQQMRGGGRGFRGGGR
RYGRRGGRRQENEEGEEE*

>Sso7d
MATVKFKYKGEEKEVDISKIKKVWRVGKMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK*

>Psp polymerase
MIIDADYITEDGKPIIRIFKKEKGEFKVEYDRTFRPYIYALLKDDSAIDEVKKITAERHGKIVRITEVEKVQKKFLGRPIEVWKLYLEHPQDVPAIREKIREHPAVVDIFEYDIPFAKRYLIDKGLTPME
GNEELTFLAVDIETLYHEGEEFGKGPIIMISYADEEGAKVITWKSIDLPYVEVVSSEREMIKRLVKVIREKDPDVIITYNGDNFDFPYLLKRAEKLGIKLPLGRDNSEPKMQRMGDSLAVEIKGRIHFDL
FPAIRRTINLPTYTLETVYEVIFGKSKEKVYAHEIAEAWETGKGLERVAKYSMEDAKVTSELGKEFFPMEAQLARLVGHPVWDVSRSSTGNLVEWFLLTKAYERNELAPNKPDEREYERRLRESYEGGYV
NEPEKGLWEGIVSLDFRSLYPSIIITHNVSPDTLNRENCKEYDVAPQVGHRFCKDFPGFIPSLLGNLLEERQKIKKRMKESKDPVEKKLLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGRQ
YIDLVRRELESRGFKVLYIDTDGLYATIPGAKHEEIKEKALKFVEYINSKLPGLLELEYEGFYARGFFVTKKKYALIDEEGKIVTRGLEIVRRDWSEIAKETQAKVLEAILKHGNVDEAVKIVKEVTEKL
SKYEIPPEKLVIYEQITRPLSEYKAIGPHVAVAKRLAAKGVKVKPGMVIGYIVLKGDGPISKRAIAIEEFDPKKHKYDAEYYIENQVLPAVERILRAFGYRKEDLRYQKTKQVGLGAWLKFMATVKFKYK
GEEKEVDISKIKKVWRVGKMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK*

>Tli polymerase
MILDTDYITKDGKPIIRIFKKENGEFKIELDPHFQPYIYALLKDDSAIEEIKAIKGERHGKTVRVLDAVKVRKKFLGREVEVWKLIFEHPQDVPAMRGKIREHPAVVDIYEYDIPFAKRYLIDKGLIPME
GDEELKLLAFDIETFYHEGDEFGKGEIIMISYADEEEARVITWKNIDLPYVDVVSNEREMIKRFVQVVKEKDPDVIITYNGDNFDLPYLIKRAEKLGVRLVLGRDKEHPEPKIQRMGDSFAVEIKGRIHF
DLFPVVRRTINLPTYTLEAVYEAVLGKTKSKLGAEEIAAIWETEESMKKLAQYSMEDARATYELGKEFFPMEAELAKLIGQSVWDVSRSSTGNLVEWYLLRVAYARNELAPNKPDEEEYKRRLRTTYLGG
YVKEPEKGLWENIIYLDFRSLYPSIIVTHNVSPDTLEKEGCKNYDVAPIVGYRFCKDFPGFIPSILGDLIAMRQDIKKKMKSTIDPIEKKMLDYRQRAIKLLANSYYGYMGYPKARWYSKECAESVTAWG
RHYIEMTIREIEEKFGFKVLYADTDGFYATIPGEKPELIKKKAKEFLNYINSKLPGLLELEYEGFYLRGFFVTKKRYAVIDEEGRITTRGLEVVRRDWSEIAKETQAKVLEAILKEGSVEKAVEVVRDVV
EKIAKYRVPLEKLVIHEQITRDLKDYKAIGPHVAIAKRLAARGIKVKPGTIISYIVLKGSGKISDRVILLTEYDPRKHKYDPDYYIENQVLPAVLRILEAFGYRKEDLRYQSSKQTGLDAWLKRMATVKF
KYKGEEKEVDISKIKKVWRVGKMISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK*

>XthA 3′-5′ exonuclease
MKFVSFNINGLRARPHQLEAIVEKHQPDVIGLQETKVHDDMFPLEEVAKLGYNVFYHGQKGHYGVALLTKETPIAVRRGFPGDDEEAQRRIIMAEIPSLLGNVTVINGYFPQGESRDHPIKFPAKAQFYQ
NLQNYLETELKRDNPVLIMGDMNISPTDLDIGIGEENRKRWLRTGKCSFLPEEREWMDRLMSWGLVDTFRHANPQTADRFSWFDYRSKGFDDNRGLRIDLLLASQPLAECCVETGIDYEIRSMEKPSDHA
PVWATFRR*

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